Segregation of COPI-rich and anterograde-cargo-rich domains in endoplasmic-reticulum-to-Golgi transport complexes

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Membrane traffic between the endoplasmic reticulum (ER) and the Golgi complex is regulated by two vesicular coat complexes, COPII and COPI. COPII has been implicated in the selective packaging of anterograde cargo into coated transport vesicles budding from the ER [1]. In mammalian cells, these vesicles coalesce to form tubulo-vesicular transport complexes (TCs), which shuttle anterograde cargo from the ER to the Golgi complex [2–4]. In contrast, COPI-coated vesicles are proposed to mediate recycling of proteins from the Golgi complex to the ER [1,5–7]. The binding of COPI to COPII-coated TCs [3,8,9], however, has led to the proposal that COPI binds to TCs and specifically packages recycling proteins into retrograde vesicles for return to the ER [3,9]. To test this hypothesis, we tracked fluorescently tagged COPI and anterograde-transport markers simultaneously in living cells. COPI predominated on TCs shuttling anterograde cargo to the Golgi complex and was rarely observed on structures moving in directions consistent with retrograde transport. Furthermore, a progressive segregation of COPI-rich domains and anterograde-cargo-rich domains was observed in the TCs. This segregation and the directed motility of COPI-containing TCs were inhibited by antibodies that blocked COPI function. These observations, which are consistent with previous biochemical data [2,9], suggest a role for COPI within TCs *en route* to the Golgi complex. By sequestering retrograde cargo in the anterograde-directed TCs, COPI couples the sorting of ER recycling proteins [10] to the transport of anterograde cargo.

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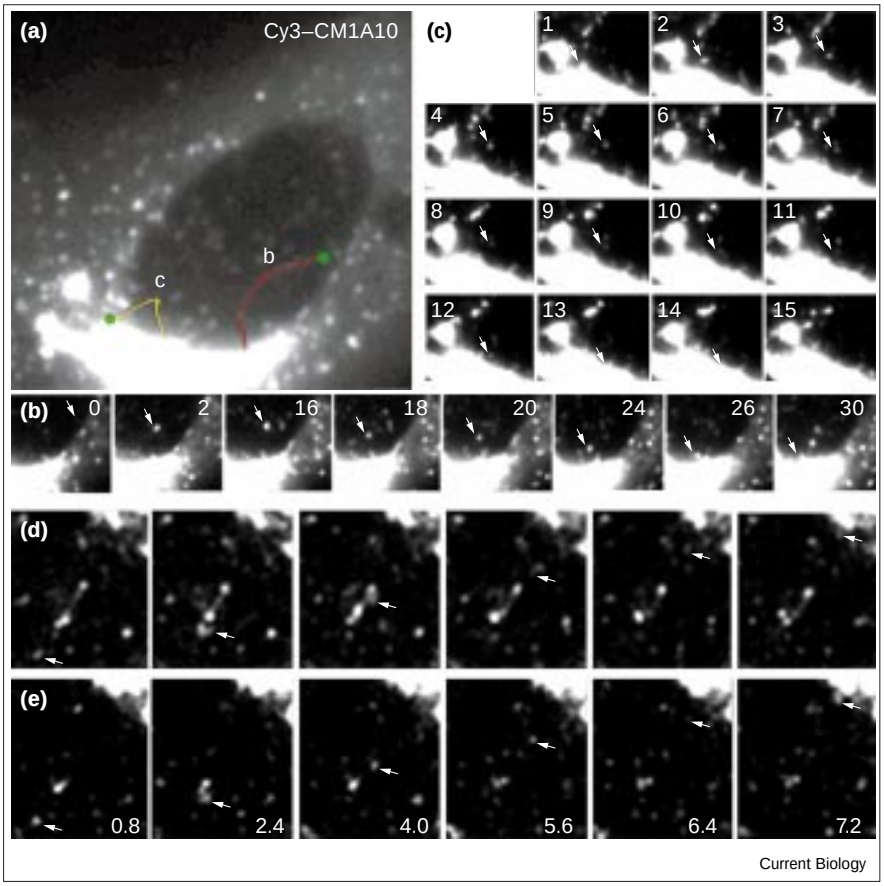
Results and discussion

Microinjection of Cy3-conjugated monovalent Fab fragments of CM1A10 [11], a monoclonal anti-COPI antibody, into living cells, or ectopic expression of ε-COP tagged with green fluorescent protein (ε-COP_{GFP}), resulted in the labelling of distinct peripheral structures that contained endogenous COPI (see Supplementary material). Neither Cy3–CM1A10 nor ε-COP_{GFP} interfered with the transport kinetics of a secretory marker, a temperature-sensitive form of the vesicular stomatitis virus glycoprotein (ts-O45-G) from the ER to the plasma membrane. Expression of ε-COP_{GFP} rescues the temperature-sensitive secretory transport block in ldlF cells caused by a point-mutation in ε-COP [12], demonstrating that ε-COP_{GFP} can functionally substitute for wild-type ε-COP (see Supplementary material). Thus, Cy3–CM1A10 and ε-COP_{GFP} can both be used as faithful markers of COPI *in vivo*.

The dynamics of fluorescently labelled COPI-containing structures in living cells were characterized and quantified using time-lapse microscopy. Of all COPI-coated structures that were detectable at the beginning of the 2 minute observation period, 34 ± 6% had saltatory movements, which lasted 1–2 seconds and had an estimated peak speed of 2–3 μm/second. Because these movements were directed towards the Golgi complex (Figure 1a,b and Supplementary material), such structures were termed anterograde-directed COPI-containing TCs. Another ~10% of the COPI-containing TCs typically underwent a series of rapid looping movements, resulting in repetitive tracking within a confined region of the cell periphery (see Supplementary material). Surprisingly, less than 5% of COPI-containing structures arose on the surface of the Golgi complex and moved towards the cell periphery, a direction that is consistent with retrograde transport. These structures almost invariably returned to the Golgi complex, usually on a distinct trajectory (Figure 1a,c and Supplementary material). In contrast to anterograde-directed COPI-containing TCs, which frequently moved long distances (> 10 μm), these retrograde-directed COPI-containing TCs travelled short distances (< 5 μm) and rarely reached the cell periphery. Anterograde, retrograde and looping COPI-containing TCs all moved at a similar average speed of around 1 μm/second. Approximately 15% of the COPI-coated structures did not move significantly (> 2 μm) within the observation period and about 35% disappeared from the focal plane, thus preventing further analysis.

The unexpected prevalence of COPI-coated structures moving towards rather than away from the Golgi complex

Figure 1



Direct visualisation of COPI and the secretory-transport marker ts-G-GFP$_{ct}$ in ER-to-Golgi transport. Vero cells were microinjected with a Cy3-conjugated anti-COPI antibody (Cy3–CM1A10) at 37°C and immediately imaged by time-lapse video microscopy, using light attenuated 50 times. Images were acquired every sec for 2 min at 37°C. **(a)** An overview of one analysed cell. Red and yellow lines indicate the trajectories of an anterograde and retrograde COPI-containing TC, respectively. Starting points are marked by green dots. Individual images of **(b)** the anterograde and **(c)** the retrograde COPI-containing TCs highlighted in (a). The arrows indicate the position of the COPI-containing TC of interest. 'QuickTime' movies (animations a–c) corresponding to this figure are available as Supplementary material. **(d,e)** Vero cells, in which ts-G-GFP$_{ct}$ was accumulated in the ER by incubation at 39.5°C for 6 h, were injected with Cy3–CM1A10 at 39.5°C. (d) Ts-G-GFP$_{ct}$ and (e) fluorescently labelled COPI were visualised by confocal microscopy at 31°C, with simultaneous image acquisition of the Cy3 and GFP images at 0.8 sec intervals. Arrows indicate a COPI-containing TC that transports ts-G-GFP$_{ct}$ to the Golgi complex.

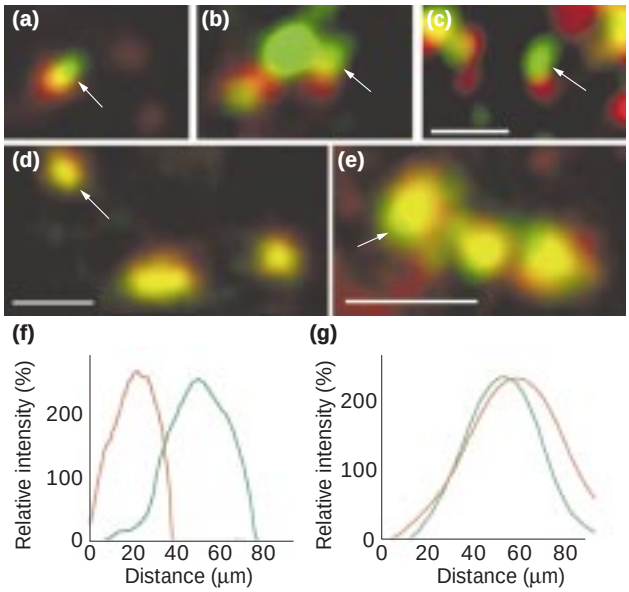
was not an artefact of Cy3–CM1A10 binding, because COPI-containing TCs labelled with ε-COP$_{GFP}$ showed identical behaviour (see Supplementary material).

To investigate the relationship between COPI-containing TCs and secretory cargo in living cells, two GFP-tagged anterograde transport markers, ts-G-GFP$_{ct}$ [13] and a truncated version of the Golgi-resident enzyme *N*-acetylglucosaminyltransferase I (NAGFP [13]), which are transported vectorially from the ER to the Golgi, were expressed and co-visualised with Cy3–CM1A10 in living Vero cells. On shifting to the permissive temperature of 31°C, ts-G-GFP$_{ct}$ rapidly accumulated in TCs that moved towards the Golgi complex, as previously described [3,4]. Over 90% of these TCs were continuously labelled with Cy3–CM1A10 during their entire journey (Figure 1d,e). In contrast, only 60% of Cy3-labelled COPI-containing TCs were also labelled with ts-G-GFP$_{ct}$ at any given time, suggesting that not all the COPI-containing TCs transport ts-G-GFP$_{ct}$, in agreement with previous results obtained in fixed cells [14,15]. Similar results were obtained using newly synthesised NAGFP, which was also rapidly transported from the ER to the Golgi complex in COPI-coated TCs (see Supplementary material).

As expected, ts-G-GFP$_{ct}$ was not present in COPI-containing TCs but was retained in the ER when cells were incubated at 39.5°C. Neither was it found in COPI-containing TCs when it was in transit from the *trans*-Golgi network to the plasma membrane, and was never found in retrograde COPI-containing TCs, suggesting that these are not directly involved in ts-G-GFP$_{ct}$ transport (data not shown).

Closer analysis of the distributions of COPI and secretory cargo in living cells revealed that ts-G-GFP$_{ct}$ often labelled the end of the moving TCs that faced the Golgi complex, whereas COPI localised predominantly to the opposite end (Figure 2a–c). By measuring the relative intensities of GFP and Cy3 fluorescence across TCs, the distribution of COPI was shown to overlap that of ts-G-GFP$_{ct}$ by, on average, 51 ± 7%, $n = 27$ (Figure 2f). This polarised distribution was more pronounced in TCs close to the Golgi complex and appeared to increase during the movement of TCs from the cell periphery (60–70% overlap) to the Golgi complex (< 30% overlap, Figure 2a,c). Similar results were obtained in fixed cells immunostained for ts-O45-G and β-COP, and when CD8 was used as the secretory marker (data not shown).

Figure 2



Distribution of COPI and membrane markers on TCs. (a–c) TCs double-labelled with ts-G-GFP$_{ct}$ (green) and Cy3–CM1A10 (red) in living cells, corresponding to time-points (a) 0.8 sec, (b) 3.2sec and (c) 6.4sec of the experiment shown in Figure 1d,e. Arrows point to the TCs of interest. (d) Confocal images of TCs in fixed cells double-labelled with antibodies against COPI (red) and the KDEL receptor (green). (e) TCs labelling positive for ts-G-GFP$_{ct}$ (green) in a living cell microinjected with Cy3–CM1A10 (red) and anti-EAGE. (f,g) Intensity profiles of the COPI and ts-G-GFP$_{ct}$ labelling on the TC marked by an arrow in (c) and (e), respectively. The green and red lines represent the profiles for GFP (ts-G-GFP$_{ct}$) and Cy3 (COPI) fluorescence, respectively. Scale bars represent 7.5 μm.

Fixed cells were co-stained with antibodies against COPI and the KDEL receptor, ERGIC 53 or p23, three membrane proteins cycling between the ER and Golgi complex. The distributions of these proteins overlapped with COPI on TCs considerably more than did ts-G-GFP$_{ct}$: 81 ± 7% for the KDEL receptor, $n = 87$; 86% ± 4 for ERGIC 53, $n = 47$; and 88% for p23, $n = 67$ (Figure 2d and data not shown). Co-staining with two distinct COPI antibodies was used as a control and these overlapped on TCs by 95 ± 4% (data not shown). These data suggest differential compartmentalisation of proteins during TC movement. Similar distributions of ERGIC 53 and syntaxin 5 relative to COPI on TCs have been previously reported in fixed cells [16,17], supporting the proposed role for the TC as a sorting organelle [18,19].

When COPI was inactivated by microinjection of an anti-β-COP antibody (anti-EAGE), which inhibits ER-to-Golgi transport [20], the dynamics of COPI-containing and secretory-marker-positive TCs were strongly inhibited (Table 1 and Supplementary material). A similar inhibition of the dynamics of COPI-containing TCs resulted when cells were treated with the microtubule-disrupting drug nocodazole for 10 minutes prior to observation (Table 1). The segregation of TCs into domains rich in either COPI or anterograde cargo was also inhibited by microinjection of the anti-EAGE antibody (Figure 2e,g) or by incubation of cells at 15°C, a temperature known to block secretory transport between the ER and Golgi complex. The overlap of COPI with ts-G-GFP$_{ct}$ on TCs was 84 ± 6% ($n = 37$) and 89 ± 4% ($n = 41$), respectively, under these conditions. Thus, it appears that the segregation of TCs into COPI-rich domains and anterograde-cargo-rich domains is closely linked to the dynamics of the TCs. It remains unclear, however, whether inhibition of TC movement results in inhibition of segregation or vice versa.

Table 1

Quantitation of the motility of COPI-containing TCs.

Treatment of cells	Number of moving COPI-containing TCs* (%)	Average displacement (μm/min)†
Control	85.3 ± 10.9	34.8 ± 4.2
Nocodazole	8.3 ± 5.2	3.3 ± 0.15
Injection of anti-EAGE‡	15.2 ± 4.2	5.1 ± 0.25

*More than 100 COPI-containing TCs in randomly chosen fields from at least three different cells were followed for 25 sec. They were scored as moving when they moved further than 2 μm or when they moved and subsequently left the plane of focus during the observation period. Data are expressed as mean ± standard deviation (SD). †The distance travelled by TCs that were followed for at least 25 sec ($n > 50$) was determined and expressed as average displacement ± SD. ‡A corresponding QuickTime movie showing an example of a cell injected with anti-EAGE is shown in the Supplementary material.

Biochemical and genetic data have provided strong evidence for the role of COPI in the retrieval of proteins from the Golgi complex to the ER [10,19,21,22], leading to the concept that COPI vesicles mediate retrograde transport from the Golgi complex to the ER [1,5–7]. To account for the localisation of COPI to COPII-containing TCs in mammalian systems, it has been suggested that COPI-containing vesicles form from these TCs and mediate retrieval of ER proteins [3,8,9]. Our data show that the predominant COPI-labelled structures observed *in vivo* are vehicles for the transfer of secretory cargo from the ER to the Golgi complex. We cannot, however, exclude the possibility that there is a more substantial pool of retrograde-directed COPI-containing vesicles, which are beyond the limits of detection in our system. But the continuous association of the COPI coat with the majority (> 90%) of anterograde-directed TCs and the functional link between COPI and ER-to-Golgi transport suggests some role for COPI on anterograde TCs.

Our observations *in vivo*, when integrated with the available biochemical and genetic data, suggest that one role for COPI on ER-to-Golgi TCs is to bind and sequester proteins destined for retrieval to the ER. This interaction results in the establishment of distinct retrograde-cargo and anterograde-cargo domains within the TC itself. Because we can provide no evidence for COPI-coated vesicles budding from these moving TCs, we suggest that following the transport of TCs to the *cis*-Golgi, anterograde cargo is delivered for further transit through the secretory pathway whereas retrograde cargo is packaged for delivery back to the ER. Establishing whether it is the retrograde-directed COPI-containing TCs, or other carriers, that are involved in vectorial transport back to the ER awaits the development of tools to visualise retrograde cargo *in vivo*.

Supplementary material

Supplementary material including QuickTime movies of the results shown in Figure 1 and Table 1 and additional methodological details is available at http://current-biology.com/supmat/supmatin.htm.

Acknowledgements

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Supplementary material

Segregation of COPI-rich and anterograde-cargo-rich domains in endoplasmic-reticulum-to-Golgi transport complexes

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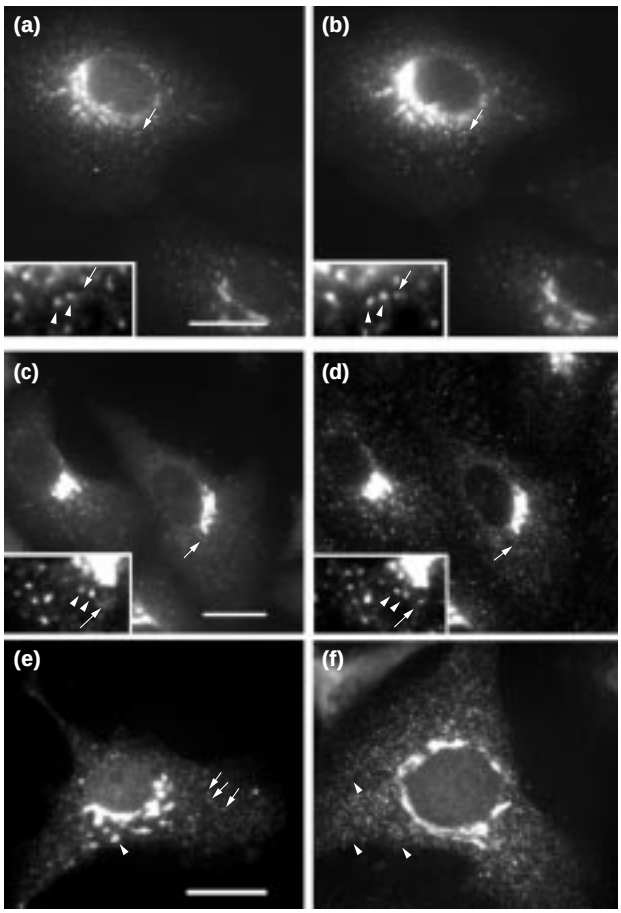
Figure S1



Fluorescent labelling of COPI in living cells. COPI was fluorescently labelled in living cells by microinjection of either (a,b,e,f) Cy3-conjugated monovalent Fab fragments of anti-COPI antibody CM1A10, or (c,d) a plasmid encoding ε-COP_{GFP}. Cells were fixed (a,b) 30 min or (c,d) 12 h after microinjection and immunostained with anti-β-COP antibodies (b,d). Arrowheads indicate overlapping structures, whereas arrows indicate non-overlapping structures. The insets show the respective areas of interest magnified 2.5 times. (e,f) Vero cells were injected with Cy3–CM1A10 either (f) with or (e) without GTPγS and fixed 10 min later. Arrows in (e) indicate TCs typically labelled by Cy3–CM1A10 in living or fixed cells. The arrowhead indicates a larger structure in the region of the Golgi complex. Arrowheads in (f) point to the numerous distinct COPI-positive structures resulting from GTPγS injection. Scale bars represent 20 μm.

Supplementary materials and methods

Cloning of ε-COP_{GFP}

Human brain ε-COP was obtained from I.M.A.G.E.consortium (40054; Genbank accession number for ε-COP is AJ13118), fused through a linker (AAEQLISEEDLP) to the amino terminus of a bright-red-shifted GFP variant [S1], and subcloned into the mammalian vector pUHD10.3 [S2].

Antibodies

Mouse monoclonal antibodies against ERGIC 53 [S3], the cytoplasmic tail of ts-O45-G (P5D4, [S4]), the KDEL receptor (anti-KDEL-R, [S5]) and CD8 [S6], and rabbit polyclonal antibodies against β-COPI (anti-EAGE [S7]) and β′-COP [S8] were used in the experiments. Mouse IgG (Cappel) was used as a control. Monovalent Fab fragments of monoclonal antibody CM1A10 (anti-COPI, [S9]; hybridomas kindly provided by Jim Rothman) were prepared as described [S7] and subsequently labelled with Cy3 (Amersham) according to the manufacturer's instructions. Labelling efficiency was approximately one fluorophore per Fab fragment. Anti-p23 antibodies were raised in rabbits as described [S10].

Quantitation of COPI dynamics and co-localisation

Quantitative analysis of the trajectories of COPI-containing TCs was performed using an extension to the IPlab software package developed at the ICRF light microscopy laboratory (obtainable from Imperial Cancer Technology Transfer Inc. (ICRT)). TCs were categorized as anterograde-directed or retrograde-directed if they exhibited directed movement with trajectories ≥ 3 μm and either terminated or were initiated within 1μm of the Golgi ribbon, respectively. The labelling density profiles of double-labelled TCs were determined using NIH image version 1.60. Raw images acquired by confocal microscopy were normalized for each colour separately. An average lowpass filter was used to remove image noise, and then the intensity profile along a line crossing the entire TC was determined for each individual colour and plotted.

Movie 1–3

Direct visualisation of COPI and the secretory transport marker ts-G-GFP_{ct} in ER-to-Golgi transport. These three movies (1–3) accompany Figure 1a–c, respectively. They are 'QuickTime' animations and are accelerated by a factor of four.

Movie 4

The dynamics of COPI-containing TCs labelled by expression of ε-COP_{GFP} in living cells. DNA encoding ε-COP_{GFP} was microinjected into the nuclei of Vero cells and the dynamics of the expressed ε-COP_{GFP} was analysed 24 h later by time-lapse microscopy.

Movie 5

The dynamics of a COPI-containing TC exhibiting rapid looping movements in the cell periphery of a live Vero cell.

Movie 6

The motility of COPI-containing TCs in live cells microinjected with an anti-β-COPI antibody (anti-EAGE). TCs were visualised using Cy3–CM1A10. The animation accompanies Table 1.

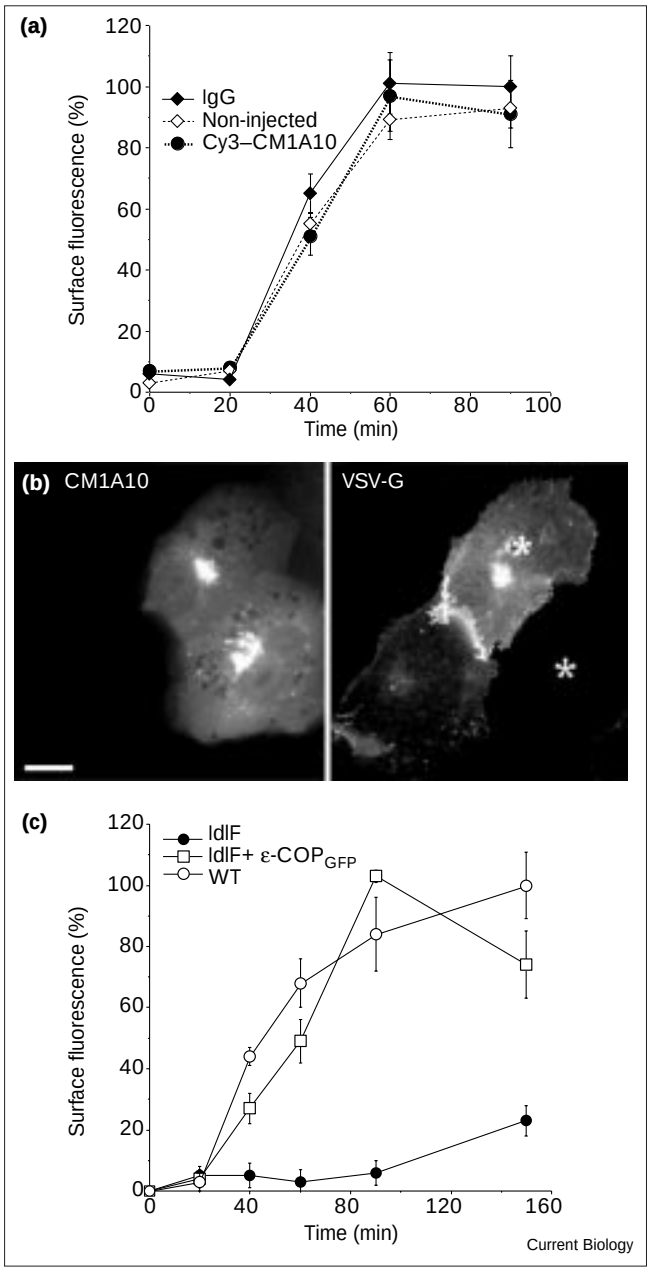


Figure S2

Rate of appearance of ts-O45G at the plasma membrane is not affected in cells containing fluorescently labelled COPI. (**a**) Vero cells were infected at 39.5°C with ts-O45 for 2.5 h, subsequently injected at 39.5°C with control mouse IgGs (filled diamonds), Cy3–CM1A10 (filled circles) or were not injected (open diamonds), and then shifted to the permissive temperature of 31°C for different times before fixation and immunostaining for cell-surface ts-O45-G. The appearance of ts-O45-G at the cell surface was quantified as described [S7,S11] and plotted as the averages ± SD of two independent experiments (n > 60 cells for each experiment); the data are normalised to the surface fluorescence in control IgG-injected cells at 60 min. (**b**) Vero cells were injected with ts-G-GFP_{ct} DNA and incubated for 6 h at 39.5°C to accumulate ts-G-GFP_{ct} in the ER. They were then injected with Cy3–CM1A10 and immediately observed by confocal time-lapse microscopy at 31°C for 3 min. Images were acquired at 1 sec intervals using light attenuated 50 times (data not shown). The cells were then left unperturbed for a further 75 min to allow ts-G-GFP_{ct} to move completely to the cell surface, fixed and then processed for surface ts-045-G fluorescence. Asterisks indicate injected cells. The scale bar represents 15 μm. (**c**) IdlF cells were injected with ts-G-GFP_{ct} either alone (filled circles), or in combination with ε-COP_{GFP} (open squares) and compared to wild-type CHO cells injected with ts-G-GFP_{ct} (open circles). Cells were incubated for 12 h at 39.5°C to allow ts-G-GFP_{ct} to accumulate in the ER and for the degradation of endogenous ε-COP in IdlF cells. The temperature was then shifted to 31°C in the presence of cycloheximide for the indicated times, and cells were fixed and immunostained for ts-G-GFP_{ct} at the plasma membrane. The data were normalised to 0% at 0 min and 100% for the brightest cell in each set of experiments. Data are expressed as mean ± SD from 2–3 independent experiments (n > 120 cells per time-point).

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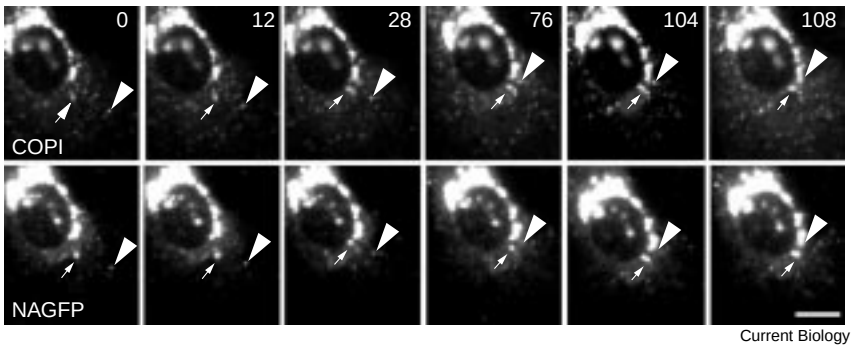
Figure S3



Simultaneous visualisation of COPI and the secretory marker NAGFP in ER-to-Golgi transport. Vero cells were microinjected with a plasmid encoding the GFP-tagged Golgi resident enzyme NAGTI (NAGFP [S12]). After 45 min, cells were injected with Cy3–CM1A10 and immediately observed by time-lapse video microscopy for 3 min. Sequential images of NAGFP and Cy3-CM1A10 were acquired every 3 sec. The arrowhead and arrow indicate two COPI-containing TCs that carry NAGFP to the Golgi complex. The scale bar represents 15 μm.

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